

*SUB A2*

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**WHAT IS CLAIMED IS:**

1. A method for selectively stimulating proliferation and differentiation of T lymphoid cells to generate a high density of clinically relevant numbers of T lymphoid cells, comprising:
  - 5 collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;
  - treating the cells are under conditions whereby *ex vivo* differentiation of the cells into Th2-like or Th2 cells is induced; and
  - 10 contacting, in the absence of exogenous interleukin-2, the material with two or more activating proteins specific for cell surface proteins present on cells in the material and in an amount sufficient to induce *ex vivo* cell expansion, whereby the cells expand to at least about  $10^{10}$  cells comprising predominantly Th2 or Th2-like cells.
2. The method of claim 1, further comprising purification of the expanded cells.
3. The method of claim 1, wherein the expanded cells are specific for a defined antigen.
4. The method of claim 1, wherein the expanded cells are predominantly Th2 cells.
- 20 5. The method of claim 1, wherein the cells are activated *ex vivo* in the presence of IL-4 with or without the presence of anti-gamma interferon and anti-IL-12 monoclonal antibodies to cause differentiation into Th2 cells.
- 25 6. The method of claim 1, wherein the immune cells are activated *ex vivo* in the presence of interferon- $\gamma$ , whereby differentiation of Th2 cells are effected.
7. The method of claim 1, wherein the proteins specific for cell surface proteins are one or more monoclonal antibodies specific for immune cell surface proteins.

8. The method of claim 7, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more of the following: CD4, CD8, CD11a, CD27, CD28, CD44 and CD45RO.

5 9. The method of claim 1, wherein cell expansion is effected in a hollow fiber bioreactor.

10. The method of claim 1, wherein the cells are expanded to an excess of  $10^{10}$  cells.

11. The method of claim 4, wherein the expanded cells are  
10 purified.

12. The method of claim 1, wherein the mammal is a human.

13. The method of claim 2, wherein the mammal is a human.

14. The method of claim 7, wherein the mammal is a human.

15. The method of claim 1, wherein the expanded cells are predominantly Th2 cells, whereby the resulting population has a Th2 or Th2-like cytokine profile.

16. The method of claim 2, wherein the expanded cells are predominantly Th2 cells, whereby the resulting population has a Th2 or Th2-like cytokine profile.

20 17. The method of claim 7, wherein the expanded cells are predominantly Th2 cells, whereby the resulting population has a Th2 or Th2-like cytokine profile.

18. A method for generating clinically relevant cell numbers of Th2 or Th2-like T lymphoid cells, comprising:

25 (a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the T lymphoid cells to alter their cytokine production profile by causing differentiation of the cells into Th2 or Th2-like cells; and

- (c) inducing cell proliferation and expanding the cells under conditions that produce at least about  $10^{10}$  cells/liter of a homogeneous population of Th2 or Th2-like T lymphoid cells.
19. The method of claim 18, wherein the T lymphoid cells with  
5 altered cytokine profile are purified.
20. The method of claim 18, wherein the T lymphoid cells with altered cytokine profile are specific for a defined antigen.
21. The method of claim 18, wherein the T lymphoid cells are activated to differentiate into Th2 cells.
- 10 22. The method of claim 18, wherein the resulting population of expanded cells includes Th2-like cells.
23. The method of claim 22, wherein the cells are activated in the presence of IL-4 anti-gamma interferon antibodies and/or anti-IL-12 antibodies, whereby cells differentiate into Th2 cells
- 15 24. The method of claim 18, wherein the cells are expanded in the presence of two or more monoclonal antibodies.
25. The method of claim 24, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more of the following: CD4,  
20 CD8, CD11a, CD27, CD28, CD44 and CD45RO.
26. The method of claim 18, wherein the cells are expanded in a hollow fiber bioreactor.
27. The method of claim 18, wherein the cells are expanded to an excess of  $10^9$  cells.
- 25 28. The method of claim 18, wherein the cells are expanded to an excess of  $10^{10}$  cells.
29. A method for generating clinically relevant numbers of regulatory T lymphoid cells for autologous cell therapy, comprising:  
30 (a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

- (b) treating the cells to induce differentiation of mononuclear cells into Th2 or Th2-like cells; and  
(c) contacting the resulting differentiated cells with two or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory cells for autologous cell therapy are generated.
- 5           30. The method of claim 29, wherein cells are purified from the material.
- 10          31. The method of claim 29, wherein the treating and contacting steps occur in the absence of exogenous cytokines or the contacting step occurs in the absence of exogenous cytokines.
32. The method of claim 29, wherein the cells are specific for a selected antigen.
- 15          33. The method of claim 29, wherein the resulting cells comprise CD4 + T-cells.
34. The method of claim 29, wherein the resulting cells are predominantly Th2 cells.
35. The method of claim 29, wherein the resulting cells comprise 20 CD8 + T-cells.
36. The method of claim 29, wherein at step (b) the cells are treated with IL-4 with or without anti-gamma interferon antibodies and/or anti-IL-12 antibodies to cause differentiation into Th2 cells.
37. The method of claim 29, wherein the proteins specific for cell 25 surface proteins are one or more monoclonal antibodies specific for immune cell surface proteins.

38. The method of claim 37, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more antigens selected from the group consisting of CD4, CD8, CD11a, CD27, CD28, CD44 and

5 CD45RO.

39. The method of claim 29, wherein cell expansion is effected in a hollow fiber bioreactor.

40. The method of claim 29, wherein the cells are expanded to about  $10^9$  cells or greater.

10 41. The method of claim 29, wherein the cells are expanded to about  $10^{10}$  cells or greater.

42. The method of claim 29, wherein the expanded cells are predominantly Th2 cells.

15 43. The method of claims 29, wherein the expanded cells are contained in a volume of one liter or less.

44. The method of claim 29, wherein the expanded cells are contained in a volume of about 500 mls or less.

45. The method of claim 29, wherein the expanded cells are contained in a volume of about 250 mls or less.

20 46. The method of claim 29, wherein the expanded cells are predominantly Th2-like cell, wherein:

Th2-like cells are cells that produce a majority of Th2 cytokines.

25 47. A method for generating clinically relevant numbers of regulatory Th2, or Th2-like lymphoid cells for autologous cell therapy, comprising:

- (a) collecting material comprising body fluid or tissue containing T lymphoid cells from a mammal;
- (b) treating the cells to induce differentiation of some of the mononuclear cells into Th2 or Th2-like cells, wherein Th2-like cells are cells that produce a majority of Th2 cytokines; and

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(c) contacting the cells with two or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of Th2 or Th2-like lymphoid cells  
5 are generated.

48. The method of claim 47, wherein cells are either purified or purged from the material.

49. The method of claim 47, wherein the treating or contacting steps occur in the absence of exogenous cytokines.

10 50. The method of claim 47, wherein the regulatory cells are specific for a defined antigen.

51. The method of claim 47, wherein the regulatory cells are CD4 + T-cells.

15 52. The method of claim 47, wherein the regulatory cells are CD8 + T-cells.

53. The method of claim 47, wherein the cells are treated with IL-4 with or without the presence of anti-gamma interferon monoclonal antibodies and/or anti-IL-12 monoclonal antibodies to cause the differentiation into Th2 cells.

20 54. The method of claim 47, wherein the proteins specific for cell surface proteins are one or more monoclonal antibodies specific for immune cell surface proteins.

55. The method of claim 54, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of 25 monoclonal antibodies specific for one or more of the following: CD4, CD8, CD11a, CD27, CD28, CD44 and CD45RO.

56. The method of claim 47, wherein cell expansion is effected in a hollow fiber bioreactor.

57. The method of claim 47, wherein the cells are expanded to an excess of  $10^9$  cells.
58. The method of claim 47, wherein the cells are expanded to an excess of  $10^{10}$  cells.
- 5 59. The method of claim 47, wherein the expanded cells are administered to a patient.
60. The method of claims 47, wherein the expanded cells are contained in a volume of about one liter or less.
- 10 61. The method of claim 47, wherein the expanded cells are contained in a volume of about 500 mls or less.
62. The method of claim 47, wherein the expanded cells are contained in a volume about 250 mls or less.
- 15 63. The method of claim 47, wherein the expanded cells are predominantly Th2 cells.
64. The method of claim 47, wherein the expanded cells are predominantly Th2-like cells.
65. The method of claim 47, wherein the expanded cells are predominantly Th2 cells.
- 20 66. The method of claim 1, wherein the  $10^{10}$  cells that are predominantly Th2 cells are produced.
67. The method of claim 66, wherein the expanded cells are administered to a patient.
68. The method of claim 1, wherein the cells are at a density of  $1 \times 10^8$  cells/ml.
- 25 69. The method of claim 1, wherein density of the cells is at least  $10^9$  cells/liter.
70. The method of claim 1, wherein density of the cells is at least  $10^{10}$  cells/liter.
- 30 71. A composition, comprising predominantly Th2 or Th2-like cells produced by the method of claim 1.

72. The composition of claim 71, wherein the cells are at a density of  $1 \times 10^8$  cells/ml.

73. A method of treatment of diseases in which a Th1 cytokine profile predominates, comprising administering the composition of claim 5 71, thereby altering the ratio of Th1/Th2 cell.

74. The method of claim 72, wherein the disease is a chronic inflammatory disease, chronic infectious diseases or an autoimmune disease.

75. The method of claim 74, wherein the disease is selected 10 from the group consisting of multiple sclerosis, rheumatoid arthritis, Crohn's Disease, autoimmune thyroid disease and inflammatory bowel disease

76. The method of claim 74, wherein the disease is selected from the group consisting of infections with human immunodeficiency 15 virus, herpes simplex virus, cytomegalovirus or hepatovirus.

77. A composition produced by the method of claim 20.

78. A method of specific immunosuppression in organ and tissue transplant procedures or to provide immunoprotection in vaccination, comprising administering the composition of claim 20.

79. The method of claim 74, wherein the disease is rheumatoid arthritis, wherein the composition is produced by a method comprising: collecting mononuclear cells from a rheumatoid arthritis patient; expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to suppress or reduce the chronic inflammatory lesions of the arthritis; and infusing the resulting composition of cells into the patient.

80. The method of claim 79, wherein the number Th2 cells is at least  $10^9$ .

81. The method of claim 79, wherein the cells are contained in a 30 volume of 1 liter or less.

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82. The method of claim 74, wherein the disease is multiple sclerosis, and the composition is produced by a method, comprising: collecting mononuclear cells from a multiple sclerosis patient; expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to ameliorate the symptoms or retard or stop the progression of multiple sclerosis; and infusing the resulting composition of cells into the patient.
83. The method of claim 82, wherein the number of cells is at least  $10^9$  cells.
84. The method of claim 82, wherein the cells are contained in a volume of 1 liter or less.
85. The method of claim 82, wherein the cells have a memory phenotype.
86. The method of claim 82, wherein the cells are specific for myelin or encephalitogenic epitopes of myelin antigens.
87. The method of claim 74, wherein the disease inflammatory bowel disease (IBD), and the composition is produced by a method, comprising: collecting mononuclear cells from an IBD patient; expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to ameliorate the symptoms or retard or stop the progression of the IBD; and infusing the resulting composition of cells into the patient.
88. The method of claim 87, wherein the number of cells is at least  $10^9$  cells.
89. The method of claim 87, wherein the cells are contained in a volume of 1 liter or less.
90. The method of claim 87, wherein the disease is Crohn's disease (CD) or ulcerative colitis (UC).

91. The method of claim 87, wherein the Th2 cells are express integrin,  $\alpha 4, \beta 7$ .

92. A method for suppression transplant rejection, comprising:

collecting mononuclear cells from a patient prior to undergoing

5 organ or tissue transplantation;

expanding the cells under conditions whereby a composition

containing an amount of Th2 cells sufficient to prevent rejection of the

transplanted organ or tissue; and

infusing the resulting composition of cells into the patient.

**10**        93. The method of claim 92, wherein the number of cells is at least  $10^9$  cells.

94. The method of claim 92, wherein the cells are contained in a volume of 1 liter or less.

95. The method of claim 92, wherein the transplanted tissue are  
15 transplanted islets of Langerhans.

96. The method of claim 92, wherein the cells are specific for the alloantigens or for an antigen unique to the transplanted tissue or organ.

**97. A method for treating insulin-dependent diabetes mellitus**

**20 (IDDM), comprising:**

collecting mononuclear cells from a patient diagnosed with IDDM or at high risk for developing IDDM;

expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to prevent or retard islet

**25** destruction; and  
infusing the resulting composition of cells into the patient.

98. The method of claim 97, wherein the number of cells

99. The method of claim 97, wherein the cells are contained in a

**30** volume of 1 liter or less.

100. Cells produced by the method of claim 1 that have a CD<sup>+4</sup> phenotype.
101. Cells produced by the method of claim 1 that have a CD<sup>+8</sup> phenotype

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